*Proc. Natl. Acad. Sci. USA*
Vol. 93, pp. 6567–6571, June 1996
Immunology

# Expression of a candidate cadherin in T lymphocytes

**(cell–cell adhesion/catenin)**

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*Communicated by K. Frank Austen, Brigham and Women's Hospital, Boston, MA, January 4, 1996 (received for review October 20, 1995)*

**ABSTRACT** **Cadherins are homotypic adhesion molecules that classically mediate interactions between cells of the same type in solid tissues. In addition, E-cadherin is able to support homotypic adhesion of epidermal Langerhans cells to keratinocytes (Tang, A., Amagai, M., Granger, L. G., Stanley, J. R. & Udey, M. C. (1993) *Nature (London)* 361, 82–85) and heterotypic adhesion of mucosal epithelial cells to E-cadherin-negative intestinal intraepithelial T lymphocytes. Thus, we hypothesized that cadherins may play a wider role in cell-to-cell adhesion events involving T lymphocytes. We searched for a cadherin or cadherins in T lymphocytes with a pan-cadherin antiserum and antisera against α- or β-catenin, molecules known to associate with the cytoplasmic domain of cadherins. The anti-β-catenin antisera coimmunoprecipitated a radiolabeled species in T-lymphocyte lines that had a molecular mass of 129 kDa and was specifically immunoblotted with the pan-cadherin antiserum. Also, the pan-cadherin antiserum directly immunoprecipitated a 129-kDa radiolabeled species from an $^{125}$I surface-labeled Jurkat human T-cell leukemic cell line. After V8 protease digestion, the peptide map of this pan-cadherin-immunoprecipitated, 129-kDa species exactly matched that of the 129-kDa species coimmunoprecipitated with the β-catenin antiserum. These results demonstrate that T lymphocytes express a catenin-associated protein that appears to be a member of the cadherin superfamily and may contribute to T cell-mediated immune surveillance.**

Cadherins are responsible for sorting cells of different lineages during embryogenesis, establishing cellular polarization, and maintaining tissue morphology (reviewed in refs. 1–4). T lymphocytes have been at the fringe of study in the cadherin field, because cadherins are classically responsible for mediating interactions between cells of the same type, whereas T cells interact with a wide variety of cell types and use other families of adhesion molecules, including immunoglobulin superfamily members, integrins, and selectins. For example, when circulating T cells encounter a site of inflammation, a series of adhesive interactions is initiated, which results in the migration of T cells across the endothelium (5). First, members of the selectin family of adhesion molecules interact with carbohydrate determinants on the lymphocyte surface, causing it to roll along the endothelium. Next, local cytokines or chemoattractants stimulate an increase in the adhesiveness of T-cell integrins for their endothelial cell ligands (e.g., $\alpha^4\beta_1$ to VCAM-1 and $\alpha^L\beta_2$ to ICAM-1), such that rolling stops and the T cell migrates across the endothelium.

In addition to their presence in the circulation, T cells are found in organized lymphoid structures, such as lymph nodes, and are diffusely distributed in the parenchyma of many tissues where they interact with a wide variety of cell types. Recent studies have begun to implicate cadherins in lymphocyte adhesion. For example, during development, murine fetal thymocytes transiently express E-cadherin, raising the possibility that they may use cadherins during interactions with thymic epithelial cells (6). In another example, E-cadherin-negative intraepithelial T cells from the intestine were found to use the $\alpha^E\beta_7$ integrin in binding to mucosal epithelial cell E-cadherin (7). However, cadherin expression by mature T cells has not been reported, and E-cadherin specifically has been shown to be absent on peripheral T lymphocytes (7). In these studies, we searched for members of the cadherin superfamily in T cells by exploiting the conserved nature of the cadherin cytoplasmic domain.

The cadherin cytoplasmic domain interacts with cytoplasmic proteins termed the catenins (α, 102 kDa; β, 88–93 kDa; and γ, 80–83 kDa; refs. 2 and 8). The presence of the cytoplasmic domain is essential to the functioning of cadherins, as deletions in this region abolish catenin binding as well as cell-to-cell adhesion (9–12). Catenins stably associate with cadherins during biosynthesis (13, 14) and are thought to mediate anchorage of cadherins to the cytoskeleton (12, 15). α-Catenin is necessary for cadherin-mediated homotypic adhesion, as E-cadherin-expressing tumor cells that lack α-catenin expression fail to form cell-to-cell contacts but can be induced to aggregate upon transfection of α-catenin (16, 17). β-Catenin is homologous to the *Drosophila* segment polarity protein armidillo, as well as the cadherin-associated protein plakoglobin, and has been shown by pulse–chase experiments to bind directly to E-cadherin (14). Plakoglobin, also termed γ-catenin, interacts more weakly with the cadherin/catenin complex and is not always coimmunoprecipitated with cadherins (18).

The structural conservation of the cadherin cytoplasmic domain exploited by the catenins has also enabled the production of pan-cadherin antisera, which interact with many members of the cadherin superfamily (19). Using the pan-cadherin antiserum as well as antisera we produced against either α- or β-catenin, we searched for cadherins in T lymphocytes. We found that mature T cells derived from several sources express a catenin-associated protein of 129 kDa at the cell surface that was also directly immunoprecipitated with a pan-cadherin antiserum. This molecule is a candidate for expanding the repertoire of adhesion molecules available to T cells.

## MATERIALS AND METHODS

**Antibodies.** Rabbit antisera reactive to α- or β-catenin were produced using proteins containing glutathione *S*-transferase (Pharmacia) fused to the C-terminal 447 amino acids of human α-catenin (20) or the full-length human β-catenin (D.L.R., unpublished data). Fusion proteins were purified from bacterial lysates with a glutathione affinity matrix (21). Rabbits were immunized by sequential subcutaneous injections in complete Freund's adjuvant. The resulting antisera were evaluated by

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both ELISA and Western blotting. Both the glutathione *S*-transferase fusion proteins and native catenins were specifically detected by their corresponding antisera in a variety of tissues and in cultured Madin–Darby canine kidney (MDCK) and human intestinal epithelial cells (CaCO2). The anti-α-catenin antiserum was designated Y4, and the anti-β-catenin antiserum was designated Y6. The anti-human E-cadherin mAb E4.6 (IgG1) was described (7). The pan-cadherin antiserum directed against the C-terminal 24 amino acids of chicken N-cadherin was described (19) and obtained from Sigma.

**Cell Culture.** The Jurkat human T-cell leukemic cell line was grown in RPMI 1640 medium with 10% calf serum (HyClone), 10 mM Hepes, 2 mM L-glutamine, 100 units/ml penicillin, 100 μg of streptomycin sulfate per ml, and 50 μM 2-mercaptoethanol in 5% $CO_2$. The human intestinal intraepithelial T-lymphocyte line 390I was isolated and cultured as described (22, 23). The breast epithelial cell line 16E6.A5 (provided by Dr. V. Band, New England Medical Center, Boston, MA) was cultured as described (24). Activated peripheral blood T lymphocytes were derived by stimulating mononuclear cells from peripheral blood with phytohemagglutinin-P (Difco) in Yssel's medium (25) containing 2 nM recombinant interleukin 2 (gift of Ajinomoto, Kawasaki, Japan), 4% (vol/vol) fetal calf serum (HyClone), and 50 μM 2-mercaptoethanol, and they were grown in 10% $CO_2$. The activated T lymphocytes were studied 7–10 days after phytohemagglutinin-P stimulation when FACS (Becton Dickinson) analysis revealed a pure $CD3^+$ cell population.

**Surface Labeling.** Live cells ($2 \times 10^7$) were surface-labeled with 1 mCi (1 Ci = 37 GBq) of $Na^{125}I$ (DuPont–New England Nuclear, Boston, MA) using lactoperoxidase as described (26). The cells were solubilized in lysis buffer containing Tris-buffered saline (TBS; 50 mM Tris, pH 7.6/140 mM NaCl) with 0.5% Triton X-100, 8 mM iodoacetamide, and 1 mM phenylmethylsulfonyl fluoride (Sigma) for 1 hr at 4°C. Lysates containing the equivalent of $4 \times 10^6$ cells were immunoprecipitated with 7–12 μl of rabbit antiserum or 1 μl of E4.6 ascites and 100 μl of rat anti-mouse kappa chain mAb 187.1 culture supernatant for optimal protein A binding. Immune complexes were then incubated with protein A–Sepharose (Pharmacia) for 1 hr at 4°C with rocking. The immunoprecipitates were washed three times with 0.5% (vol/vol) Triton X-100 in 0.5 M NaCl with 50 mM Tris and two times with 0.5% Triton X-100, 0.5% deoxycholic acid, and 0.05% SDS in TBS, eluted with sample buffer (10% glycerol/3% SDS/0.5 M Tris, pH 6.8), and analyzed by SDS/7% PAGE as described (27).

**Metabolic Labeling.** Exponentially growing T cells ($2 \times 10^7$) were incubated with 4 ml of methionine- and cysteine-free RPMI 1640 medium (Select-Amine kit; GIBCO, Grand Island, NY) supplemented with 10% dialyzed fetal calf serum and 20 mM Hepes, pH 7.4 (labeling medium), for 20 min at 37°C in a T25 tissue culture flask. Alternatively, washed, exponentially growing, 85–90% confluent, 16E6.A5 cells were incubated with 4 ml of methionine- and cysteine-free RPMI 1640 medium for 20 min at 37°C in a T75 tissue culture flask. Next, 1 mCi of [$^{35}$S]methionine and [$^{35}$S]cysteine (EXPRE$^{35}$S$^{35}$S; DuPont–New England Nuclear, Boston, MA) was added, and the cells were labeled for 4 hr. The cells were solubilized in lysis buffer containing 0.5% Triton X-100. Precipitated material was separated by SDS/7% PAGE and visualized with standard fluorographic procedures (28).

**Peptide Mapping.** Jurkat cells were surface-labeled as described above. Next, the cells were lysed, precleared, and immunoprecipitated with the anti-β-catenin antiserum, Y6. Protein was eluted into 40 μl of sample buffer and loaded into 1-cm wells of a 5-cm long stacking gel. Next, 40 μl of sample buffer containing 0.06 μg of V8 protease per ml (ICN) was added. Samples were run two-thirds of the way into the stacking gel and the current was turned off for 30 min to allow digestion into peptides, which were then resolved on a SDS/12% polyacrylamide gel.

**Western Blotting.** Cell equivalents ($2 \times 10^7$) were immunoprecipitated with the anti-β-catenin antiserum Y6 or preimmune serum and washed, eluted, and electrophoresed by SDS/PAGE as described above. Proteins were transferred to a poly(vinylidene difluoride) membrane in 20% (vol/vol) methanol, 0.05% (wt/vol) SDS, 3 g of Tris per liter, and 14.3 g of glycine per liter for 4 hr at 300 mA. The membrane was blocked in 2% (wt/vol) gelatin in TBS for 1 hr at room temperature, washed twice in TBS/T (TBS containing 0.2% Tween 20), and incubated with a 1/5000 dilution of the pan-cadherin antiserum in TBS/T for 2 hr at room temperature. The membrane was then washed six times with TBS/T before being incubated with peroxidase-conjugated protein A for 1 hr at room temperature. Excess peroxidase-conjugated protein A was removed by washing six times with TBS/T. Pan-cadherin reactive protein was visualized using enhanced chemiluminiscence (ECL; Amersham) with an exposure time of 12 sec.

## RESULTS

To search for the presence of cadherins in T lymphocytes, a β-catenin-reactive rabbit antiserum was produced and used to identify coimmunoprecipitating proteins in T-cell lysates in comparison with epithelial cell lysates known to contain E-cadherin. 16E6.A5 epithelial cells and Jurkat T cells were solubilized in 0.5% Triton X-100 and immunoprecipitated with control preimmune antiserum or the anti-human β-catenin antiserum, Y6. The eluted and reduced proteins were sepa-

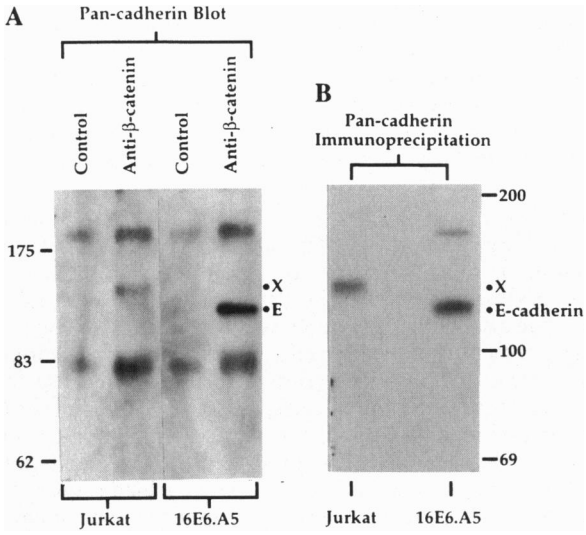


FIG. 1. T cells express a β-catenin-associated protein that is pan-cadherin-reactive and expressed at the cell surface. (*A*) 16E6.A5 epithelial cells and Jurkat T cells were solubilized and immunoprecipitated with control preimmune antiserum or the anti-β-catenin antiserum (Y6), reduced with 2-mercaptoethanol, and resolved on SDS/7% PAGE. Proteins were transferred to poly(vinylidene difluoride), and the membrane was blotted with the anti-pan-cadherin antiserum (Sigma). After visualization with ECL, a 123-kDa protein was visualized from 16E6.A5 epithelial cells (E-cadherin), and a 129-kDa protein was visualized from Jurkat T cells (X). Relative molecular masses are shown in kDa. (*B*) In parallel, anti-pan-cadherin precipitations were carried out on aliquots of 16E6.A5 cells and Jurkat T cells that had been surface-labeled with $^{125}$I. The isolated proteins were separated on the same gel as in *A*. A 123-kDa protein was visualized from 16E6.A5 epithelial cells (E-cadherin), and a 129-kDa protein was visualized from Jurkat T cells (X), indicating that the blotted protein species comigrated with the surface-labeled protein. Molecular masses are shown in kDa.

rated by SDS/7% PAGE, transferred to a poly(vinylidene difluoride) membrane, and blotted with the pan-cadherin antiserum. The 16E6.A5 cell line contained one β-catenin-associated, pan-cadherin-reactive species at 123 kDa (consistent with the size of E-cadherin) that was absent from the control lane, whereas Jurkat cells contained one labeled species at 129 kDa not seen in the control lane (Fig. 1*A*, X). In parallel, separate aliquots of each cell line were surface-labeled with $^{125}$I, directly precipitated with the pan-cadherin antiserum, and resolved on the same polyacrylamide gel used for blotting in Fig. 1*A*. In Jurkat cells, a radiolabeled species of 129 kDa was seen and 16E6.A5 cells contained a 123-kDa species consistent with the size of E-cadherin (Fig. 1*B*). The surface radiolabeled proteins from both cell types (Fig. 1*B*) comigrated with the protein coimmunoprecipitated with β-catenin in the pan-cadherin blot (Fig. 1*A*). In addition, 16E6.A5 cells contained a second surface-labeled species of unknown identity at 166 kDa (Fig. 1*B*), which was recognized by the pan-cadherin antiserum. As the catenins are intracellular proteins, they are not visualized by this technique. Members of the cadherin superfamily range in size from 120 to 130 kDa. Thus, the 129-kDa β-catenin-associated protein in T cells was a candidate cadherin. Surface staining of these T cells was negative for both E- and P-cadherin (data not shown).

Two additional non-tumor T-cell lines, activated adult peripheral blood T lymphocytes and the *in vitro*-cultured interleukin 2-dependent intraepithelial T-cell line 390I, were surface-labeled with $^{125}$I, solubilized, immunoprecipitated with the control preimmune serum and the anti-β-catenin antiserum Y6, and analyzed by SDS/PAGE in parallel with the Jurkat T-cell line. In all three T-cell lines, a 129-kDa surface-radiolabeled species was coprecipitated with the anti-β-catenin antiserum (Fig. 2).

To confirm the identity of the coimmunoprecipitating species seen in the anti-β-catenin immunoprecipitations with material directly immunoprecipitated with the pan-cadherin antiserum, $^{125}$I-labeled Jurkat T cells were solubilized in Triton X-100, immunoprecipitated with antisera directed against α-catenin (Y4), β-catenin (Y6), and the pan-cadherin antiserum, and visualized by SDS/PAGE. The 129-kDa putative cadherin expressed by Jurkat T cells was visualized with all three antisera (Fig. 3*A*). In parallel, additional aliquots of the $^{125}$I-labeled Jurkat lysate used in Fig. 3*A* were immunoprecipitated with either the anti-β-catenin or the pan-cadherin antisera and subjected to Cleveland digest peptide mapping. After the proteins were eluted from the protein A sepharose beads, they were digested with V8 protease, and the resulting peptides were separated by SDS/PAGE. The array of peptides generated by both antisera was identical (Fig. 3*B*), indicating that the 129-kDa radiolabeled species seen in anti-β-catenin coimmunoprecipitations was almost certainly the same protein as the 129-kDa species that was directly precipitated by the pan-cadherin antiserum.

To visualize the catenins associated with the putative cadherin in T cells, 16E6.A5 epithelial cells and activated adult peripheral blood T lymphocytes were biosynthetically labeled, solubilized in 0.5% Triton X-100 detergent, and immunoprecipitated with control preimmune serum or the anti-α-catenin antiserum, Y4. SDS/PAGE analysis under reducing conditions revealed a radiolabeled species at 102 kDa, the expected size of α-catenin, in both cell types (Fig. 4). In addition, in 16E6.A5 cells, three other prominent species were seen at 123, 93, and 81 kDa, which are the expected sizes for E-cadherin, β-catenin, and γ-catenin, respectively (Fig. 4). A similar pattern of proteins was seen in anti-E-cadherin (mAb = E4.6) immunoprecipitates (data not shown). The anti-α-catenin immunoprecipitation from activated peripheral blood T lymphocytes contained a prominent catenin-associated species larger than that seen in 16E6.A5 cells at 129 kDa (designated X) and an additional radiolabeled species at 93 kDa (β-catenin; Fig. 4). Activated peripheral blood T lymphocytes did not contain a species identifiable as γ-catenin at 81 kDa, as seen in the 16E6.A5 epithelial cells.

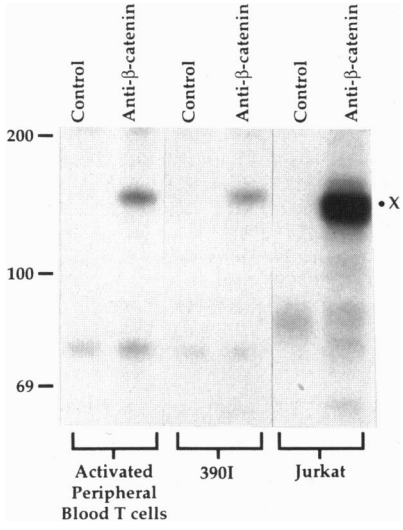


FIG. 2. A 129-kDa β-catenin-associated protein is expressed by both non-tumor and leukemic T cell lines. Activated peripheral blood T cells (9 days after phytohemagglutinin-P stimulation), the intraepithelial T cell line 390I, and Jurkat cells were surface-labeled with $^{125}$I, solubilized, and precipitated with control preimmune antiserum, or the anti-β-catenin antiserum (Y6), reduced with 2-mercaptoethanol, and run on SDS/7% PAGE. All three T-cell lines expressed a protein at 129 kDa, indicating that the catenin-associated protein is present in non-tumor as well as leukemic T cells. Molecular masses are shown in kDa.

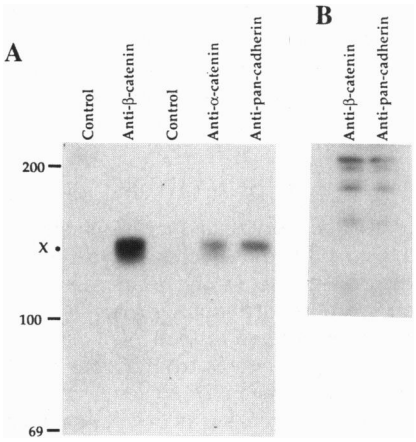


FIG. 3. The catenin-associated protein in T cells is identical to the protein directly recognized by a pan-cadherin antiserum. (*A*) Jurkat cells were surface-labeled with $^{125}$I, solubilized with Triton X-100, immunoprecipitated with control preimmune antiserum, the anti-β-catenin antiserum (Y6), the anti-α-catenin antiserum (Y4), or the anti-pan-cadherin antiserum (Sigma), reduced with 2-mercaptoethanol, and resolved on SDS/7% PAGE. All three antisera immunoprecipitated a protein of 129 kDa, indicating that the pan-cadherin-reactive catenin-associated protein either associated both α-catenin and β-catenin or exists in a complex containing both α-catenin and β-catenin. Molecular masses are shown in kDa. (*b*) Jurkat cells were surface-labeled with $^{125}$I, solubilized in Triton X-100 and precipitated with the anti-β-catenin antiserum (Y6) or the pan-cadherin antiserum. Eluted proteins were digested with 0.6 μg of V8 protease per ml and electrophoresed two-thirds of the way into the stacking gel before the current was turned off for 30 min to allow protease digestion. Peptides were then separated in a 12% polyacrylamide gel. Note that the β-catenin-associated protein and the pan-cadherin-reactive protein generated identical peptide maps.

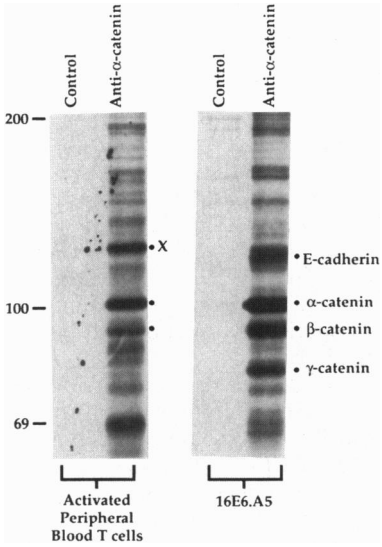


FIG. 4. Demonstration of an α-catenin-associated protein in T cells. 16E6.A5 epithelial cells and activated peripheral blood T lymphocytes were metabolically labeled with [$^{35}$S]methionine and [$^{35}$S]cysteine for 4 hr, solubilized, immunoprecipitated with control preimmune antiserum or the anti-α-catenin antiserum (Y4), reduced with 2-mercaptoethanol, and analyzed by SDS/7% PAGE. In each cell line, a radiolabeled species can be visualized at 102 kDa, corresponding to the directly precipitated α-catenin. 16E6.A5 epithelial cells express E-cadherin, the α-catenin-associated protein present in epithelial cells at 123 kDa, whereas a 129-kDa radiolabeled species is seen in the T-cell lysates (X, for candidate cadherin "X"). Both lysates contain a protein at 93 kDa the size of β-catenin, but only the 16E6.A5 epithelial cells have a protein at 81 kDa, the size of γ-catenin. Molecular masses are shown in kDa.

Together these data demonstrate that mature T lymphocytes grown in suspension cultures express a putative cadherin that is expressed at the cell surface and recognized by a pan-cadherin antisera as well as coprecipitated with antisera that recognize either α- or β-catenin.

## DISCUSSION

To search for members of the cadherin superfamily in T cells, we exploited the fact that most cadherins are complexed to the ubiquitously expressed α-, β-, and γ-catenins via a conserved region in their cytoplasmic domain. A pan-cadherin antiserum as well as antisera generated to recognize either α- or β-catenin were used to screen lysates from T cells for the presence of proteins in the size range of the cadherins (120–130 kDa). Using both metabolic and surface labeling techniques, we found that T cells expressed a pan-cadherin-reactive, α- and β-catenin-associated protein that was a candidate cadherin based on its relative molecular weight of 129 kDa. Reactivity with the pan-cadherin reagent suggested that the 129-kDa protein was a member of the cadherin superfamily. The identity of the catenin-associated protein with the pan-cadherin-reactive protein was confirmed by peptide mapping. As the T cells used in these experiments do not express E-cadherin (refs. 6 and 7; data not shown for Jurkat), it remains possible that the 129-kDa protein identified here is another previously described cadherin, a new member of the cadherin superfamily, or a non-cadherin catenin-associated protein.

Precedent for the existence of non-cadherin, catenin-associated proteins is accumulating from immunoprecipitation analysis of epithelial cells with antibodies to E-cadherin, α-catenin, or β-catenin where proteins other than α-catenin, β-catenin, or plakoglobin can be visualized (29). One such protein is the adenomatous polyposis coli (APC) gene product, a cytoplasmic protein that associates with β-catenin and plakoglobin (30–32). In addition, β-catenin can associate with the epidermal growth factor receptor (33) and c-erbB-2 (34). Thus, the 129-kDa catenin-associated protein in T cells is likely to be a cadherin, because it is in the size range of cadherins, is expressed at the cell surface, and reacts with a pan-cadherin antiserum; however, at the present time, we cannot rule out the possibility that it may be unrelated to the cadherin superfamily.

The description of a catenin-associated candidate cadherin in T cells (distinct form E-cadherin) may expand the repertoire of cell adhesion molecules used by T cells, and does not change our previous conclusion that T cell-expressed $\alpha^E\beta_7$ binds to epithelial cell E-cadherin. As most organs express cadherins in a tissue-restricted manner, T cells may use their cadherin in the recognition of solid tissues that express the same cadherin. Such interactions might mediate lymphocyte localization to specific tissue sites and mediate immune surveillance. T cells expressing cadherins may also use them in interactions with other T cells or with other leukocytes expressing cadherins. Screening T cells isolated from different tissue sites for the presence of a cadherin will determine if the expression of cadherins by T cells is dependent on their microenvironment. Given the important role for cadherins in cellular adhesion and signaling, the identification of cadherin expression on T cells portends an important role for these molecules in cellular immunity.

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